Vol. 111, No. 3 December, 1956

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

A NEW PHYCOERYTHRIN FROM PORPHYRA NAIADUM [1]

R. L. AIRTH [2] AND L. R. BLINKS

Hopkins Marine Station of Stanford University, Pacific Grove, California

Svedberg and Katsurai (1929) proposed a phylogenetic nomenclatural system for the classification of the phycobilin pigments of the algae. They designated the phycoerythrin and phycocyanin from the red algae as R-phycoerythrin and R-phycocyanin, respectively, and the corresponding pigments from the blue-green algae as C-phycoerythrin and C-phycocyanin. In general, these pigments exhibit the following absorption maxima:

Pigment	Approximate absorption maxima mμ			
R-phycoerythrin	495	540	560	
C-phycoerythrin		550		
R-phycocyanin		550		615
C-phycocyanin				615

This system has proved inadequate in several instances (Kylin, 1912; Lemberg, 1930; Kylin, 1940; Haxo, *et al.*, 1955) in that phycobilin pigments other than the above types, as judged from their absorption spectra, have been isolated. In spite of the apparent shortcomings of the Svedberg and Katsurai system of classification, no new system has been proposed.

A new phycoerythrin has now been isolated from *Porphyra naiadum* and it is proposed that this pigment be called B-phycoerythrin (tentatively so designated by Blinks, 1954). This differs from known phycoerythrins in having two absorption peaks, at 545 and 565 mμ. The isolation, purification and some properties of this pigment will be discussed.

Source

Porphyra naiadum Anderson is a member of the most primitive red algal order, the Bangiales. There is now some question as to whether it belongs in the genus *Porphyra,* since its life cycle is different. This is currently under study by Prof. G. J. Hollenberg; pending his description of a new genus, we must use the current name. The thallus is one cell thick, extremely delicate, and yields its pigment readily into fresh water in a few hours. It is found growing only upon a marine flowering plant, *Phyllospadix.*

[1] Research supported under contract with the Office of Naval Research (No. NR 120-050).

[2] Present address: McCollum-Pratt Institute, The Johns Hopkins University, Baltimore 18, Maryland.

Materials and Methods

Masses of thalli were stripped from the host plant and washed with distilled water. The washed algal mass was then just covered with distilled water and kept at 5° C. for about 15 hours. The supernatant, which contains the water-soluble phycobilin pigments, was separated from the algae by centrifugation at 15,000 times gravity for one hour. By this procedure approximately 75 per cent of the total phycobilins present can be extracted. The supernatant was filtered twice through Whatman No. 1 filter paper and the filtrate centrifuged at 20,000 *g* for twenty minutes. The pH of the pigment solution at this stage was 6.8–7.0. The pigment solution was then dialyzed in "Visking" tubing for 12 hours at 1° C. against 0.1 *M* acetate buffer, pH 5.0. The dialyzed pH 5.0 pigment solution constituted the stock solution and will, in the future, be referred to as such.

Results and Discussion

Purification and crystallization

The general method of purification and crystallization of phycobilin pigments, which has varied slightly from investigator to investigator, involved precipitation with ammonium sulfate (Kylin, 1912; Kitasato, 1925; Lemberg, 1928). This precipitation is carried out after the algae have been extracted for several days at room temperature under slightly basic conditions.

This procedure was attempted on freshly extracted stock pigment solution from *P. naiadum:* it consistently failed to crystallize phycoerythrin and phycocyanin although concentrated ammonium sulfate precipitated an amorphous mass. If the stock pigment solution was allowed to stand at room temperature for several days, then phycoerythrin (but not phycocyanin) could be crystallized by this method. Bannister (1954) working with the blue-green alga *Synechocystis* apparently encountered similar difficulties in trying to crystallize with a freshly extracted pigment solution. It seems very likely that the phycoerythrin obtained by the classical procedure may be a modified pigment.

In contrast to ammonium sulfate treatment a freshly prepared stock pigment solution yielded well-formed phycoerythrin crystals on simply standing, in the cold, for about 24 hours at pH 4.5 These crystals were separated by centrifugation, washed in acetate buffer at pH 4.5, and redissolved in water adjusted to pH 7.5. Recrystallization could be carried out by reacidification to pH 4.5. The first crystallization gave an estimated 20 per cent yield of the total phycoerythrin present, while the second and subsequent recrystallizations were quantitative. The reason for this low yield in the original crystallization will be discussed in a subsequent communication. (Suffice it to say here that the non-crystallizable fraction appears to have an iso-electric point (if at all) in very acid ranges.)

The absorption spectra of the original crystals and three-times recrystallized B-phycoerythrin are presented in Figure 1. The main absorption maximum is at 545 mμ; this value did not vary between pH 5.0 to 7.0. Phycoerythrin that is kept at pH 9.0 for 6 hours has the same absorption maximum but the blue (400–450 mμ) and red (600–700 mμ) absorption is increased by about 300 per cent. Recently Haxo *et al.* (1955) have found that the phycoerythrin from *Porphyridium* was modified above pH 7.3. Our findings are also consistent with theirs in that

we find that phycocyanin from *P. naiadum* is irreversibly bleached at pH 9.0. In contrast to the phycoerythrin from *Porphyridium*, however, neither the native nor the crystalline B-phycoerythrin from *Porphyra naiadum* tended to form additional "shoulders" or absorption maxima under alkaline conditions.

In addition, it is apparent that the original B-phycoerythrin crystals exhibit a "shoulder" in the 565 mμ spectral region which is lacking in the three-times recrystallized preparation. Whether this absorption represents an impurity in the original crystallization or a modification of phycoerythrin on repeated recrystalliza-

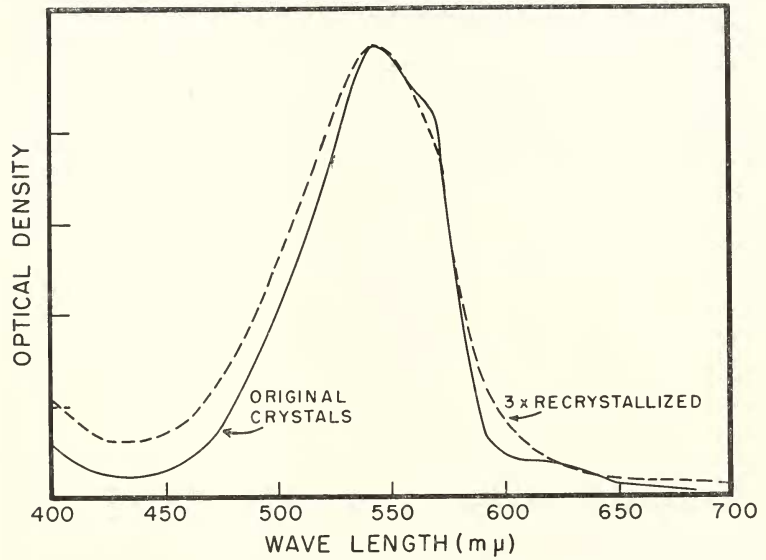

FIGURE 1. Absorption curves of once- and three-times recrystallized B-phycoerythrin. Solid line, original crystals; dashes, three-times recrystallized.

tion is unknown at the present time. There are, however, reasons for believing that the latter may be the case. On repeated recrystallization it was found that the B-phycoerythrin solubility at pH 7.5 decreased with the number of times the pigment was recrystallized. Also the stability of the native and crystalline phycoerythrin to hydrogen peroxide is different. Table I gives the reduction in optical density at 540 mμ of the two pigment solutions when treated with varying hydrogen peroxide concentrations. The pH of both solutions was 5.8.

B-phycoerythrin was also isolated by the chromatographic method of Swingle and Tiselius (1951). Haxo *et al.* (1955) have successfully separated the phycobilin pigments, including allophycocyanin, from several algal species by this method. The latter pigment is also present in *P. naiadum* and this was the only method we found to isolate allophycocyanin and phycocyanin in relatively pure form.

The order of pigment elution from the column depends upon the pH of the elutant. Using 1 to 2 *M* acetate buffer at pH 5.0 as an elutant, the pigments come off the column in the following order: phycocyanin, B-phycoerythrin, a mixture of phycoerythrin and phycocyanin. Allophycocyanin could not be eluted from the column at pH 5.0; however, it was eluted with 0.1 *M* phosphate buffer, pH 7.0. When phosphate buffer (0.05 to 0.1 *M*), pH 7.0, was used the elution order of the pigment was: B-phycoerythrin, a mixture of phycoerythrin, phycocyanin and allophycocyanin, and finally allophycocyanin.

The phycoerythrin isolated by this method was, however, never completely free of phycocyanin and repeated chromatography did not remove this impurity.

Ultracentrifugation

Svedberg and his collaborators determined the molecular weight of crystalline phycoerythrin from several species of red algae (Svedberg and Lewis, 1928; Svedberg and Katsurai, 1929; Svedberg and Eriksson, 1932). In a final paper of this series Eriksson-Quensel (1938) found the molecular weight of R-phycoerythrin

TABLE I

The reduction in optical density (at 540 mμ) of stock pigment solution and crystalline B-phycoerythrin, treated with H_2O_2 for four hours

Per cent H_2O_2	Stock pigment solution $-\Delta E_{540}$	Crystalline B-phycoerythrin $-\Delta E_{540}$
0	0.040	0.000
1	0.874	+0.204
3	1.146	0.000
5	1.060	0.000

from *Ceramium rubrum* to be 290,000 between pH 3.0 to 10.0. At other pH values the R-phycoerythrin molecule breaks down into units with a molecular weight of 34,600 or a multiple thereof.

In view of the fact that we were dealing with a new type of phycoerythrin it was considered desirable to determine its molecular weight and compare it with that of R-phycoerythrin. The ultracentrifugation experiments were carried out for us through the very kind courtesy of Drs. H. Cook and J. M. Luck of the Department of Chemistry, Stanford University.

B-phycoerythrin that had been recrystallized several times was dissolved at pH 7.0, centrifuged, and dialyzed at 1° C. in 0.05 *M* acetate buffer, pH 5.0. The sedimentation constant ($s_{20} \times 10^{13}$) was determined on the dialyzed preparation in a Spinco ultracentrifuge and found to be 12.0. This value is comparable to that found by Eriksson-Quensel (1938). Consequently it seems very probable that the molecular weights of R- and B-phycoerythrin are the same. The crystalline B-phycoerythrin was judged to be homogeneous from the fact that only one schlieren peak was observed and the absorption boundary of the pigment corresponds almost exactly with it. It may also be noted that Svedberg and Eriksson (1932) determined the molecular weight of what they designated native R-phycoerythrin from *Ceramium* and found it to be similar to the crystalline phycoerythrin from this species.

Electrophoresis

The mobility of crystalline B-phycoerythrin was tested by dissolving it at pH 7.5, centrifuging and then dialyzing at 1° C. in acetate buffer of 0.1 ionic strength, pH 5.0. The movement is toward the anode at the rate of about 2.0×10^{-5} cm.^{2}/sec./volt.

The above value was determined by visually measuring the movement of the ascending and descending boundary in a Tiselius apparatus (no schlieren optics were available). Fairly reproducible results could be obtained, because of the intense color of the pigment.

Crystalline B-phycoerythrin had charge characteristics different from those of the remainder of the pigments in the stock solution and it was possible to determine

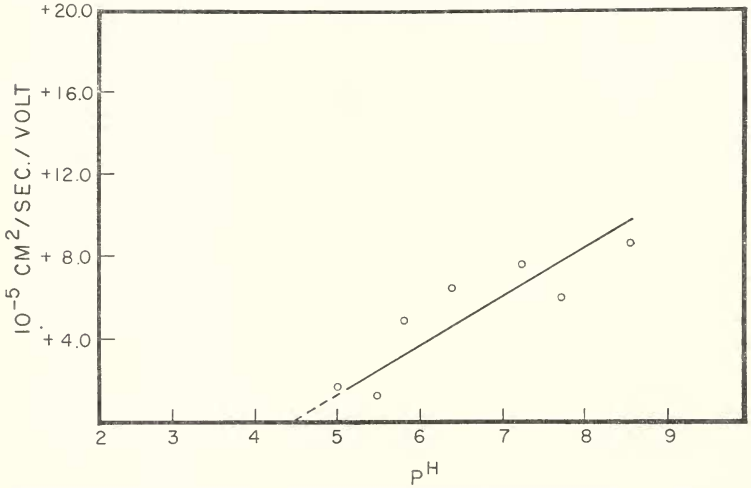

FIGURE 2. pH-mobility curve of crystalline B-phycoerythrin. Mobility in 10^{-5} cm.^{2}/sec./volt. Determinations by visual measurement in Tiselius apparatus.

the pH-mobility characteristics of this pigment. In this case the mobility values were determined from either the ascending or descending boundary, depending upon the pH of the determination. The pH-mobility curve of B-phycoerythrin is presented in Figure 2. No values were determined below pH 5.0 as the pigment tended to precipitate below this pH value. The isoelectric point as extrapolated by this method would be approximately at pH 4.5 which corresponds well with that found for crystallization.

Other optical properties

The fluorescence spectrum of B-phycoerythrin has been determined by French, Smith, Virgin and Airth (unpublished data); at pH 7.0 the fluorescence maximum is at 578 mμ.

The phycobilin constituents of *P. naiadum* are B-phycoerythrin, phycocyanin and allophycocyanin. At least the two former pigments are photosynthetically active in that they pass absorbed light energy on to chlorophyll. In such studies it is often essential to know the percentage of the total light absorbed by each pigment at various wave-lengths. As purified pigments were available a curve analysis of the absorption of the stock pigment solution was carried out. The percentage of the total light absorbed by each of the phycobilin pigments at their respective absorption maxima is presented in Table II. These values are fairly consistent with those presented by Yocum and Blinks (1954).

TABLE II

Percentage absorption of the total light absorbed by the various phycobilin pigments at different wave-lengths of an extracted pigment solution of Porphyra naiadum

Wave-length mμ	Pigment	Per cent total light absorbed
545	B-phycoerythrin	88
	phycocyanin	9
	allophycocyanin	3
615	B-phycoerythrin	2
	phycocyanin	77
	allophycocyanin	21
655	B-phycoerythrin	0
	phycocyanin	18
	allophycocyanin	82

The authors gratefully acknowledge the many helpful suggestions of Dr. C. B. van Niel during the course of this work.

SUMMARY

A new phycoerythrin, B-phycoerythrin, isolated from *Porphyra naiadum*, is described. The purification and crystallization, ultracentrifugation, electrophoretic and optical properties of this pigment are discussed. It has a major absorption peak at 545 mμ with a minor, and transient, one at 565 mμ, which tends to disappear on repeated crystallization. The molecular weight is apparently the same as that of R-phycoerythrin (ca. 290,000). Its iso-electric point is close to pH 4.5, and its mobility (toward the anode) at pH 5.0 is about 2×10^{-5} cm.^{2}/sec./volt.

LITERATURE CITED

BANNISTER, T. L., 1954. Energy transfer between chromophore and protein in phycocyanin. *Arch. Biochem. Biophys.*, **49**: 222–233.

BLINKS, L. R., 1954. The role of accessory pigments in photosynthesis. In: Symposium on Autotrophic Micro-organisms. Cambridge, England; Cambridge University Press.

ERIKSSON-QUENSEL, I., 1938. The molecular weights of phycoerythrin and phycocyanin. *Biochem. J.*, **32**: 585–589.

HAXO, F., C. O'HEOCHA AND P. NORRIS, 1955. Comparative studies of chromatographically separated phycoerythrins and phycocyanins. *Arch. Biochem. Biophys.*, **54**: 162–173.

KITASATO, Z., 1925. Biochemische Studien über Phykoerythrin und Phykocyan. *Acta Phytochima (Japan)*, **11**: 75–97.

KYLIN, H., 1912. Über die roten und blauen Farbstoffe der Algen. *Zeitschr. f. Physiol. Chem.*, **76**: 397–425.

KYLIN, H., 1940. Über Phykoerythrin und Phykocyan bei *Ceramium rubrum*. *Zeitschr. f. Phys. Chem.*, **69**: 169–239.

LEMBERG, R., 1928. Die Chromoproteide der Rotalgen. *Ann. Chemie* (*Liebig*), **461**: 46–89.

LEMBERG, R., 1930. Chromoproteide der Rotalgen II. Spaltung mit Pepsin und Saüren. Isolierung eines Pyrrolfarbstoffs. *Ann. Chemie* (*Liebig*), **477**: 195–245.

SVEDBERG, T., AND I. B. ERIKSSON, 1932. The molecular weights of phycocyanin and phycoerythrin. *J. Amer. Chem. Soc.*, **54**: 3998–4010.

SVEDBERG, T., AND I. T. KATSURAI, 1929. The molecular weights of phycocyanin and of phycoerythrin from *Porphyra tenera* and of phycocyanin from *Aphanizomenon flos aquae*. *J. Amer. Chem. Soc.*, **51**: 3573–3583.

SVEDBERG, T., AND N. B. LEWIS, 1928. The molecular weights of phycoerythrin and of phycocyanin. *J. Amer. Chem. Soc.*, **50**: 525–536.

SWINGLE, S. M., and A. TISELIUS, 1951. Tricalcium phosphate as an adsorbent in the chromatography of proteins. *Biochem. J.*, **48**: 171–174.

YOCUM, C. S., AND L. R. BLINKS, 1954. Photosynthetic efficiency of marine plants. *J. Gen. Physiol.*, **38**: 1–16.